



Evidence of metyrapone reduction by two Mycobacterium strains shown by ^1H NMR

Bruno Combourieu, Pascale Pascale Besse, P. Besse-Hoggan Besse-Hoggan,
Martine Sancelme, Edmund Maser, A.M. Delort

► To cite this version:

Bruno Combourieu, Pascale Pascale Besse, P. Besse-Hoggan Besse-Hoggan, Martine Sancelme, Edmund Maser, A.M. Delort. Evidence of metyrapone reduction by two Mycobacterium strains shown by ^1H NMR. Biodegradation, 2004, 15, pp.125-132. 10.1023/B:BIOD.0000015625.94368.a5 . hal-00136049

HAL Id: hal-00136049

<https://hal.science/hal-00136049>

Submitted on 12 Mar 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Evidence of metyrapone reduction by two *Mycobacterium* strains shown by ^1H NMR

Bruno Combourieu¹, Pascale Besse¹, Martine Sancelme¹, Edmund Maser² & Anne-Marie Delort^{1*}

¹Laboratoire de Synthèse et Etude de Systèmes à Intérêt Biologique, UMR 6504 du CNRS, Université, Blaise Pascal, 63177 Aubière Cedex, France; ²Department of Experimental Toxicology, Faculty of Medicine, University of Kiel, D-24105 Kiel, Germany (*author for correspondence: e-mail: amdelort@chimie.univ-bpclermont.fr)

Key words: metyrapone, metyrapol, *Mycobacterium*, *in situ* ^1H NMR, reductases

Abstract

In situ ^1H NMR monitoring of metyrapone incubations with resting-cells of two strains of *Mycobacterium*, *Mycobacterium aurum* MO1 and *Mycobacterium* sp. RP1, showed the biotransformation of this compound, and more precisely the carbonyl-reduction of metyrapone into the corresponding alcohol, metyrapol. This reduction produced both enantiomers. The use of inhibitors allowed us to show the multiple enzymatic activities involved in this biotransformation including carbonyl reductase (EC 1.1.1.184) from the short-chain dehydrogenase superfamily and aldehyde reductase (EC 1.1.1.2) from the aldo-keto reductase superfamily.

Introduction

Carbonyl reductases belong to the oxido-reductase enzymes and are able to transform a wide range of aliphatic and aromatic xenobiotic carbonyl compounds, in particular into the corresponding alcohols. These reduction reactions are carried out in main by members of two distinct superfamilies, the short-chain dehydrogenase/reductase superfamily (SDR) and the aldo-keto reductase superfamily (AKR). SDR members are soluble proteins, mostly dimers or tetramers, containing about 270 amino acids for the monomer and a characteristic catalytic triad (Ser, Tyr and Lys) in the active site (Jornvall et al. 1999; Kallberg et al. 2002; Oppermann et al. 2003). The AKR members are usually monomeric, NAD(P)H dependent, about 320 amino acids in length with an active site containing a conserved tetrad (Tyr, His, Asp and Lys) (Jez et al. 2001; Hyndman et al. 2003). Both AKR and SDR seem to be the result of a convergent evolution in that many enzymes of these superfamilies exhibit pluripotency for steroidal and non-steroidal carbonyl substrates (Maser 1995). Carbonyl reducing enzymes

can accept many structurally different substrates (endogenous as well as xenobiotics: pharmaceuticals agents or toxicologically important derivatives), but they can be distinguished by the substrates they use and their specific inhibitors (Forrest & Gonzalez 2000; Oppermann & Maser 2000).

The presence of carbonyl reductases has been reported in various organisms, but the majority of the research work was performed in mammalian cells and tissues, their essential role being the protection from damage by the accumulation of toxic carbonyl compounds. They can thus participate in detoxication processes (Maser & Oppermann 1997; Atalla & Maser 2001; Finckh et al. 2001).

Considering microorganisms, carbonyl reduction was mainly investigated by chemists, the aim of their studies being the production of optically active alcohols (Besse et al. 1997; Roberts 1997; Faber 1998). If the enantiomeric excess obtained for the alcohol is not good enough, authors concluded that either the enzyme involved is not enantioselective, or the reduction is the result of multiple enzymatic activities. Therefore, the class of the enzyme involved

in such reaction was often unclear. Only a few papers report serious work on the purification, identification and characterization of carbonyl reductases and aldehyde reductases from microorganisms: *Saccharomyces cerevisiae* (Ford & Ellis 2001, 2002), *Sporobolomyces salmonicolor* and *Candida magnoliae* (Kita et al. 1999), *Geotrichum candidum* (Matsuda et al. 2000), *Rhodococcus erythropolis* (Zelinski et al. 1994), *Escherichia coli* (Habrych et al. 2002). However, reduction of carbonyl xenobiotics by microorganisms can have a great importance for biotechnological uses (Ellis 2002) or in the environmental field.

Recently, we have studied the biodegradative pathway of morpholine and analogues (heterocyclic compounds) by two *Mycobacterium* strains in more detail using *in situ* ^1H NMR (Besse et al. 1998; Combourieu et al. 1998, 2000; Poupin et al. 1998; Delort & Combourieu 2001). This technique, both qualitative and quantitative, allows the study of molecules at natural abundance directly in the incubation medium without any previous step of purification. We have shown that the first step of biodegradation proceeded via an oxidation in α -position of the heteroatom (nitrogen) by a cytochrome P450 catalyzed reaction. The involvement of such an enzyme was evidenced by the use of metyrapone, a selective cytochrome P450 inhibitor. *In situ* monitoring of this inhibitory reaction by ^1H NMR revealed that metyrapone **1**, an aromatic ketone, was reduced to metyrapol **2** (Figure 1). As this type of reduction is commonly used to test carbonyl reductase activity (Maser & Netter 1989, 1991), this observation prompted us to investigate further the presence of such an enzyme in these *Mycobacterium* strains.

Materials and methods

Chemicals

Metyrapone, *p*-nitrobenzaldehyde (*p*-NBA) and tris-[3-(trifluoromethyl-hydroxymethylene)-*d*-camphorato] europium III ($\text{Eu}(\text{tfc})_3$) were purchased from Aldrich Chemical (St Quentin Fallavier, France) and tetradeuterated sodium trimethylsilylpropionate (TSPd₄) was purchased from EurisoTop (St Aubin, France).

Growth conditions

Mycobacterium aurum MO1 and *Mycobacterium* sp. RP1 cultures were grown in 100 mL of Trypcase soy broth (bioMérieux, Marcy l'Etoile, France) in 500-mL

Erlenmeyer flasks incubated at 30 °C with agitation at 200 rpm. They were harvested after 48 h of culture.

Incubation with xenobiotics

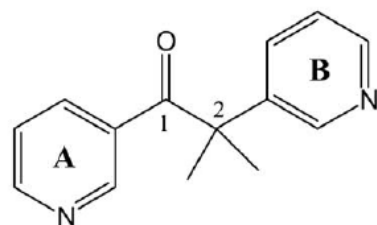
Cells were harvested by centrifugation at $9,000 \times g$ for 15 min at 5 °C. The supernatant was eliminated and the pellet was washed twice with phosphate buffer (containing per liter of distilled water, KH_2PO_4 1 g, K_2HPO_4 1 g, FeCl_3 4 mg and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 40 mg, pH 6.6) and finally resuspended in this buffer (5g of wet cells in 50 mL of buffer). The cells were incubated with 5 mM of metyrapone or *p*-nitrobenzaldehyde in 500 mL Erlenmeyer flask at 30 °C with agitation (200 rpm). Incubation of cells under the same conditions in the absence of the substrate constituted a negative control, as did incubation of the substrate in the buffer without cells. Samples (1 mL) were taken regularly. They were centrifuged at $12,000 \times g$ for 5 min. The supernatants were isolated and immediately frozen until NMR analysis.

^1H NMR spectroscopy

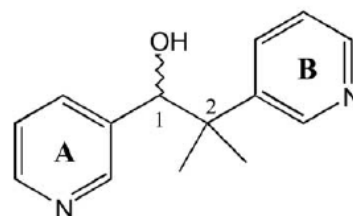
(i) *Preparation of NMR samples.* The supernatant (540 μL) was supplemented with 60 μL of a 8 mM solution of TSPd₄ in D_2O and adjusted to pH 10 with 4N NaOH. pH adjustment avoided changes in chemical shifts. D_2O was used for locking and shimming. TSPd₄ constituted a reference for chemical shifts (0 ppm) and quantification.

(ii) *^1H NMR spectra.* ^1H NMR was performed at 300.13 MHz on a Bruker Avance 300 spectrometer at 21 °C with 5 mm-diameter tubes containing 600 μL of sample. About 150 scans were collected (90° pulse, 7 μs ; relaxation delay, 5 s; acquisition time, 4.561 s; 32000 data points). No filter was applied before Fourier transformation but a baseline correction was performed on spectra before integration with Bruker software. Under these conditions, the limit of quantification was in the range of 0.05 mM.

(iii) *Quantification of metabolites.* The concentration of metabolites was calculated as follows: $[\text{m}] = (9 \times A_0 \times [\text{TSPd}_4]) / (b \times A_{\text{ref}})$, where $[\text{m}]$ is the concentration of metabolite, A_0 is the area of metabolite *m*, A_{ref} is the area of reference resonance in the ^1H NMR spectrum, *b* is the number of protons of metabolite *m* in the signal integrated, and 9 is the number of protons resonating at 0 ppm.



1 – Metyrapone



2 – (+/-)-Metyrapol

Figure 1. Structure of the compounds studied.

Synthesis of racemic metyrapol

To a solution of 1 g (4.4 mmol) of metyrapone in 40 mL of absolute ethanol was added 62.8 mg (1.7 mmol) of NaBH₄. The mixture was stirred overnight at room temperature. Ethanol was then evaporated under vacuum and the residue was diluted with 25 mL of a mixture water/HCl 0.1N 4/1 v/v. This aqueous phase was extracted three times with chloroform and the organic layer was dried on MgSO₄. After evaporation of the solvent, racemic metyrapol was obtained quantitatively. RMN ¹H (400.13 MHz, CDCl₃) δ ppm: 1.28 (s, 3H, CH₃); 1.32 (s, 3H, CH₃); 4.58 (s, 1H, CHOH); 6.07 (s, 1H, OH); 7.05 (ddd, 1H, H-5, J = 0.8 Hz, J = 4.8 Hz, J = 7.9 Hz); 7.12 (ddd, 1H, H-5', J = 0.8 Hz, J = 4.8 Hz, J = 8.0 Hz); 7.23 (ddd, 1H, H-4, J = 1.6 Hz, J = 2.3 Hz, J = 7.9 Hz); 7.53 (ddd, 1H, H-4', J = 1.5 Hz, J = 2.5 Hz, J = 8.0 Hz); 8.11 (dd, 1H, H-2, J = 0.8 Hz, J = 1.7 Hz); 8.24 (dd, 2H, H-6, J = 1.6 Hz, J = 4.8 Hz); 8.38 (dd, 1H, H-2', J = 0.8 Hz, J = 2.1 Hz). RMN ¹³C (100.62 MHz, CDCl₃) δ ppm: 23.4 (CH₃); 24.6 (CH₃); 41.8 (C(CH₃)₂); 78.4 (CHOH); 122.3 (C-5); 122.5 (C-5'); 135.1 (C-4'); 135.3 (C-4); 137.5 (C-3); 141.2 (C-3'); 146.5 (C-6'); 147.7 (C-6); 148.2 (C-2'); 148.5 (C-2).

Synthesis of the (–)-metyrapol

The optically pure (–) enantiomer of metyrapol was obtained by chemical resolution with (–)-O,O-di-*p*-toluoyltartaric acid according to Howe & Moore (1971). mp = 107–108 °C. $[\alpha]_D^{25} = -30.1$ (c = 1, EtOH), ee > 98%; Lit: $[\alpha]_D^{21} = -42.3$ (c = 1, EtOH) (Howe and Moore 1971); $[\alpha]_D^{21} = -29.0$ (c = 0.7, EtOH), ee \geq 98% (Nagamine et al. 1997). Spectral data were identical to those of the racemic mixture.

Enzymatic assays

Enzyme activities were assessed by HPLC as described previously (Maser & Bannenberg 1994; Oppermann & Maser 1996): Final concentrations: NADH 3.2 mM; NADPH 3.2 mM, NADPH-regenerating system: NADP⁺ 0.8mM; glucose-6-phosphate 6mM, glucose-6-phosphate dehydrogenase 0.35U, MgCl₂ 3 mM; carbonyl substrate: 1 or 5mM. Reversed phase HPLC conditions: Octadecyl-Si 100 polyol 4.5 mm \times 25 cm (Serva, Heidelberg, Germany); eluent: 30% acetonitrile (v/v) in 30mM phosphate buffer pH 7.4; λ = 254 nm.

Western blot analysis

Electroblotting was performed in a semi-dry blotting system. Proteins were transferred to a nitrocellulose membrane and antigen-antibody complexes were visualized by chemiluminescence (ECL PLUSTM, detection system, Amersham Pharmacia Biotech). Primary antisera were diluted 1/40,000, the secondary antibody (peroxidase conjugated swine anti-rabbit immunoglobulin, DAKO) was used in a 1/5,000 dilution. Under these conditions, the detection limit of our antibody preparation was determined at around 5 ng of 3 α -HSD/CR protein.

Results and discussion

Metyrapone reduction by Mycobacterium strains

Kinetics of metyrapone transformation were followed by *in situ* ¹H NMR spectroscopy, performed directly on the incubation medium after centrifugation. By studying the spectra obtained at different times of incubation, we could observe the decrease of the signals corresponding to metyrapone and the appearance of new signals. In the aromatic region, most of the signals

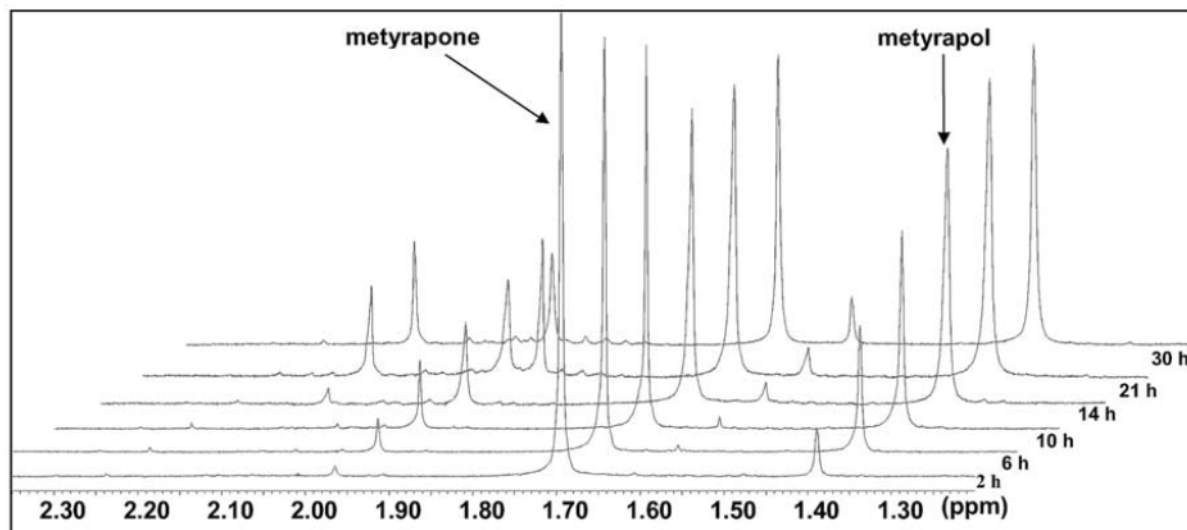


Figure 2. *In situ* ^1H NMR monitoring of metyrapone biotransformation (1.7 ppm) and metyrapol formation (1.4 ppm) by resting cells of *Mycobacterium aurum* MO1 at different incubation times.

overlap, giving difficulties for their identification. The evolution of the different compounds is more visible on the expanded region between 1.2 and 2.3 ppm of the spectra obtained at different incubation times (Figure 2).

The singlet at 1.7 ppm, corresponding to the methyl groups of metyrapone, is decreasing whereas new signals appeared, in particular a very intense singlet at 1.4 ppm, which corresponds to the major metabolite. The very similar shape and chemical shifts of the signals of this metabolite with those of metyrapone seems to evidence a close chemical structure. In order to eliminate the signals of by-products and to identify unambiguously the structure of the major metabolite, the supernatant, obtained after centrifugation of a 30 h incubation medium of metyrapone with *M. aurum* MO1, was extracted with chloroform and analysed by ^1H and ^{13}C NMR spectroscopy. The comparison of the spectra obtained with those reported in the literature (Pospisil et al. 1994) allowed us to identify the major metabolite as metyrapol, the compound being the result from reduction of the carbonyl function in metyrapone. This structure was also confirmed by mass spectrometry (Chemical Ionization): MS (CI) $m/z = 229$, $[\text{M} + \text{H}]^+$.

Quantitative analysis of the kinetics of biotransformation of metyrapone was performed by integrating the singlet of metyrapone and metyrapol at 1.7 and 1.4 ppm respectively, directly on the ^1H NMR spectra : the measured areas were compared to the

integral of TSPd₄ signal (reference) in order to calculate the different concentrations of metabolite and the parent molecule (Figure 3). The transformation of metyrapone (5mM) into metyrapol was not complete and reached a plateau after 20 h of incubation, corresponding to a 50% disappearance of metyrapone (formation of 2.2 mM of metyrapol). The formation of other metabolites was also observed as unidentified signals were present on the ^1H NMR spectra. In the literature, some authors (Damani et al. 1979, 1981; De Graeve et al. 1979) report the formation of several N-oxide derivatives during the metabolism of metyrapone by rats due to a pyridine N-oxidase. However, in our case, the sum of the substrate and product concentration decreases only very slightly during the incubation period, showing that other pathways of metyrapone metabolism do not seem to be important with *Mycobacterium aurum* MO1.

The ability of another strain of the same genus, *Mycobacterium* sp. RP1, to reduce metyrapone (5 mM) was also tested. Metyrapol was the major metabolite observed, but the kinetics of the biotransformation were much slower with this strain: 35 h of incubation are needed to reduce 50% of metyrapone.

Enantioselectivity of metyrapone reduction

Reduction of ketones by microorganisms is known to be achieved with varying degrees of enantioselectivity. The stereochemical mechanism of metyrapone reduc-

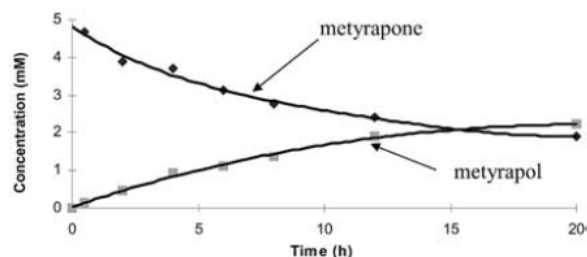


Figure 3. Time courses of the concentrations of metyrapone and metyrapol during the incubation of metyrapone (5 mM) with resting cells of *Mycobacterium aurum* MO1.

tion has already been studied in a few cases: *In vivo* metabolism of metyrapone by humans (Chiarotto & Wainer 1995), rats (Nagamine et al. 1997) or filamentous fungus (Howe & Moore 1971) or *in vitro* biotransformation by rat liver extracts (Nagamine et al. 1997) yielded the corresponding alcohol with poor to excellent enantiomeric excess. In order to study the stereoselectivity of the reductive metabolism of metyrapone by *Mycobacterium* strains, racemic and optically active metyrapol were synthesized as references. Racemic metyrapol was obtained by a borohydride reduction of metyrapone in a quantitative yield. The optically pure (–) enantiomer of metyrapol was obtained by chemical resolution with (–)-O,O-di-*p*-toluoyltartaric acid according to Howe & Moore (1971).

The enantiomeric excess of metyrapol formed during the incubation of metyrapone with the *Mycobacterium* strains was determined by ^1H NMR after adding a chemical shift reagent, an europium derivative: tris-[3-(trifluoromethyl-hydroxymethylene)-*d*-camphorato] europium (III) (Günther 1994). With an equal weight of metyrapol and of the europium derivative (Figure 4A), two proton signals of one pyridine ring were duplicated, each peak corresponding to one enantiomer. To assign the signals to each enantiomer, the same experiment was carried out with the synthesized optically pure (–)-enantiomer (Figure 4B). Figure 4C represents the expanded spectrum obtained after the addition of the europium derivative to a chloroformic extract of a supernatant, corresponding to a 96 h incubation of *Mycobacterium aurum* MO1 with 5 mM of metyrapone.

The ratio (+)/(–)-metyrapol, determined by the integration of the corresponding peak around 8 ppm, was about 50/50 whatever the used *Mycobacterium* strain was. Reduction of metyrapone by *Mycobacterium* sp. RP1 or by *M. aurum* MO1 yielded racemic metyrapol.

No enantioselectivity was observed with these strains, indicating the involvement either of a sole enzyme with a non-enantiogenicity activity, or of several carbonyl reducing enzymes.

Enzyme(s) involved in the reduction of metyrapone

Several carbonyl metabolizing enzymes are able to reduce the carbonyl moiety of a compound to the corresponding hydroxy derivative. To get an idea of the type of enzyme involved in the case of metyrapone reduction in *Mycobacterium* strains, selective inhibitors were added to the incubation medium. This method is well-known to distinguish some AKRs from carbonyl reducing enzymes of the SDR type. Quercitrin, a flavonoid inhibitor of the SDR carbonyl reductase (EC 1.1.1.184) and phenobarbital, an inhibitor of the AKR aldehyde reductase (EC 1.1.1.2) were chosen (Oppermann & Maser 2000).

The first studies on metyrapone reduction (5 mM) were carried out with resting cells of *Mycobacterium aurum* MO1 in the presence of quercitrin (5 mM) or phenobarbital (5 mM). Both inhibitors had an effect on the rate of metyrapone biotransformation. Half of metyrapone was transformed after 25 h of incubation in the presence of quercitrin instead of 9 h in its absence. Accordingly, the transformation rate of metyrapone was reduced by more than a factor of 2 in the presence of 5 mM of quercitrin, indicating the potential involvement of carbonyl reductase (SDR) in this reaction. A similar effect was also observed in the presence of phenobarbital (reduction of the transformation rate by a factor of 2), indicating the participation of aldehyde reductase in metyrapone reduction. No significant difference was observed between both inhibitors, except that the equilibrium between metyrapone and metyrapol was reached after 24 h upon inhibition by quercitrin and after 28 h upon inhibition by phenobarbital. This may indicate a slightly higher activity of aldehyde reductase rather than carbonyl reductase in metyrapone reduction in *Mycobacterium aurum* MO1. With *in situ* ^1H NMR analysis, we could observe that neither quercitrin, nor phenobarbital was transformed by *Mycobacterium* strains. Their concentrations remained stable during the incubation (data not shown).

These results clearly showed the presence of enzymes acting as carbonyl reductases in *Mycobacterium* strains. However, they do not allow us to precisely define the extent of contribution of the reductase (carbonyl reductase or aldehyde reductase) involved

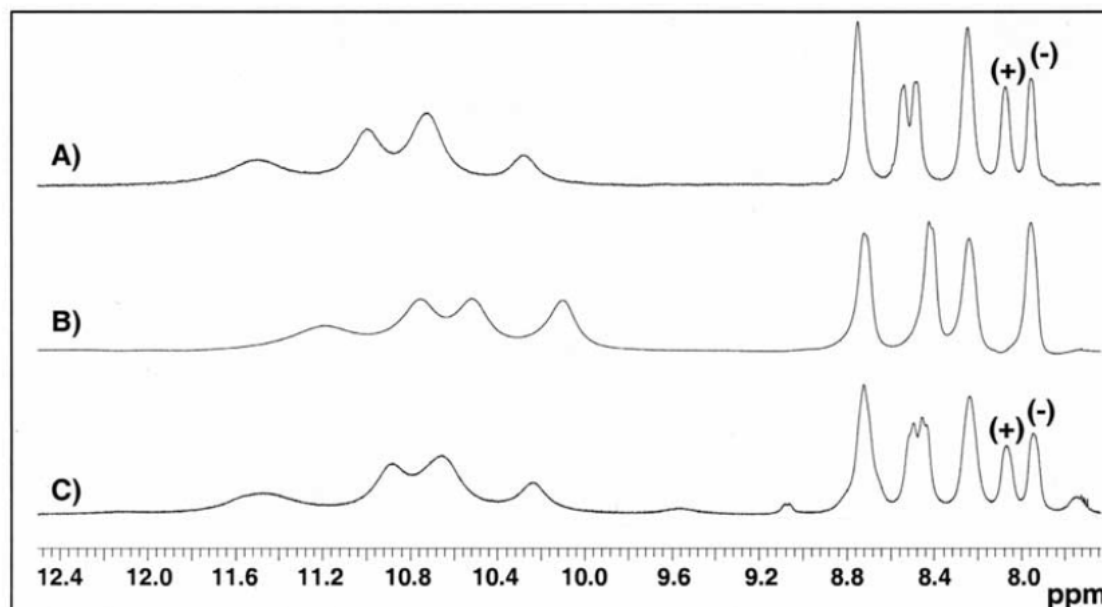


Figure 4. Expanded region of the ^1H NMR spectra obtained after addition of the europium complex of racemic metyrapol (4A), (-)-metyrapol (4B) and chloroformic extract of metyrapol obtained after a 96 h incubation of metyrapone (5 mM) with *Mycobacterium aurum* MO1 (4C).

Table 1. Assays of *p*-NBA carbonyl reduction with cell extracts from *Mycobacterium aurum* MO1 and *Mycobacterium* sp. RP1. V_{max} : nmol/mg/min, K_m : mM, K_i : mM (Inhibitor: quercitrin)

Strain	V_{max}	K_m	V_{max}/K_m	K_i
RP1	349	0.47	743	1.20
MO1	305	0.45	678	0.18

in metyrapone reduction. One of the problems is that working *in vivo* with whole-cell microorganisms, the effect of an inhibitor could be influenced by its limited access to the intracellular enzyme machinery. In addition, the potency of a selective inhibitor may be affected by the presence of other enzymes. However, even with cell extracts, the differentiation between these two carbonyl reducing enzymes is not always easy, as shown by Maser et al. (1991a, b) in the case of human and rat liver microsomes.

Some preliminary assays were carried out with cell-extracts. Immunoblot results did not reveal the presence of a *SDR*-like 3α -hydroxysteroid dehydrogenase which has previously been shown to catalyze the carbonyl reduction of metyrapone in *Comamonas testosteroni* (Oppermann & Maser 1996). Hence, other *SDRs* or *AKRs* seem to be involved. Cell extracts

of *Mycobacterium aurum* MO1 and *Mycobacterium* sp. RP1 were incubated with metyrapone, showing low activities with this substrate (for example: $K_m = 10.72$ mM; $V_{\text{max}} = 0.609$ nmol/mg of protein per min in the case of *Mycobacterium* sp. RP1). Metyrapone formation occurred with either NADPH or NADH as cofactor, although the activity with NADPH was weaker. Another well-studied substrate of carbonyl reducing enzymes, *p*-nitrobenzaldehyde (*p*-NBA) was also tested with cell extracts. *p*-NBA has been shown to be a substrate of other metyrapone reducing enzymes (Maser & Bannenberg 1994). However, since the sterical demand of metyrapone is higher than that of *p*-NBA, the latter might be substrate of other carbonyl reductases as well. *p*-NBA was a better substrate for the *Mycobacterium* strains giving higher affinities (Table 1). Even if both electron donors were accepted, a strong preference for NADPH was observed with *p*-NBA. Assays in the presence of quercitrin as inhibitor showed a different behaviour of both strains with a K_i value seven times higher in the case of *Mycobacterium* RP1. This result again demonstrates the multiplicity of bacterial reductases in the *Mycobacterium* strains.

Conclusions

Metyrapone, the diagnostic cytochrome P450 inhibitor, is reduced at its ketone function to the correspond-

ing alcohol metabolite, metyrapol, by two strains of *Mycobacterium*, *Mycobacterium aurum* MO1 and *Mycobacterium* sp. RP1. This biotransformation, clearly evidenced by *in situ* ^1H NMR performed directly on the incubation medium, is mediated by at least two enzymes, carbonyl reductase (EC 1.1.1.184) and aldehyde reductase (EC 1.1.1.2), as shown by the use of specific inhibitors. The multiplicity of reductases in these bacteria, which is further substantiated by the use of *p*-NBA as a substrate and the resulting difference in sensitivity towards quercitrin, could also explain why the reduction took place without any enantiospecificity. A way to determine more precisely which reductases are involved in metyrapol formation, it would be interesting in the future to purify the enzymes from these strains, or to screen the known genome sequence of another *Mycobacterium* strain, *Mycobacterium tuberculosis* (Cole et al. 1998) for homologies with known reductases (Kallberg et al. 2002). In fact, these enzymes – and the microorganisms having these enzymes – may have a great importance in the environmental field, and particularly in detoxification processes: the compounds formed are more hydrophilic, can be easily excreted and are generally less toxic than the parent carbonyl compound.

Acknowledgement

The authors are grateful to Dr N. Truffaut for the gift of the strain *Mycobacterium* sp. RP1.

References

- Atalla A & Maser E (2001) Characterization of enzymes participating in carbonyl reduction of 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) in human placenta. *Chem-Biol. Interact.* 130–132: 737–748
- Besse P, Bolte J, Demuyne C, Hecquet L & Veschambre H (1997) Recent progress in the “de novo” formation of asymmetric carbons by bioconversion methodology. In: Pandalai SG (Ed) *Recent Research Developments in Organic Chemistry*, Vol 1 (pp 191–228). Transworld Research Network
- Besse P, Combourieu B, Poupin P, Sancelme M, Truffaut N, Veschambre H & Delort AM (1998) Degradation of morpholine and thiomorpholine by an environmental *Mycobacterium* involves a cytochrome P450. Direct evidence of the intermediates by *in situ* ^1H -NMR. *J. Mol. Biocat. B: Enz.* 5: 403–409
- Chiarotto JA & Wainer IW (1995) Enantiospecificity of the aldo-keto reductase-mediated reduction of metyrapone in volunteers: an initial observation of an inter-individual difference. *Pharm. Sci.* 1: 79–81
- Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry III CE, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton S, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S & Barrell BG (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 396: 190–198
- Combourieu B, Besse P, Sancelme M, Godin JP, Monteil A, Veschambre H & Delort AM (2000) Common degradative pathways of morpholine, thiomorpholine and piperidine by *Mycobacterium aurum* MO1: evidence from ^1H -nuclear magnetic resonance and ionspray mass spectrometry performed directly on the incubation medium. *Appl. Environ. Microbiol.* 66: 3187–3193
- Combourieu B, Besse P, Sancelme M, Veschambre H, Delort AM, Poupin P & Truffaut N (1998) Morpholine degradation pathway of *Mycobacterium aurum* MO1: direct evidence of intermediates by *in situ* ^1H nuclear magnetic resonance. *Appl. Environ. Microbiol.* 64: 153–158
- Damani LA, Crooks PA & Cowan DA (1981) Metabolism of metyrapone. 2-Chromatographic and mass spectral properties of the N-oxides of metyrapone and metyrapol. *Biomed. Mass Spectrom.* 8: 270–277
- Damani LA, Crooks PA & Gorrod JW (1979) A high performance liquid-chromatographic method for the determination of in vitro metabolites of metyrapone. *J. Pharm. Pharmacol.* 31: 94P
- De Graeve J, Gielen JE, Kahl GF, Tutenberg KH, Kahl R & Maume B (1979) Formation of two metyrapone N-oxides by rat liver microsomes. *Drug Metab. Dispos.* 7: 166–170
- Delort AM & Combourieu B (2001) *In situ* ^1H -NMR study of the biodegradation of xenobiotics: application to heterocyclic compounds. *J. Industr. Microbiol. Biotechnol.* 26: 2–8
- Ellis EM (2002) Microbial aldo-keto reductases. *FEMS Microbiol. Lett.* 216: 123–131
- Faber K (Ed) (1998) *Biotransformations in Organic Chemistry*. 2nd Edition. Springer-Verlag, Berlin
- Finckh C, Atalla A, Nagel G, Stinner B & Maser E (2001) Expression and NNK reducing activities of carbonyl reductase and 11 β -hydroxysteroid dehydrogenase type 1 in human lung. *Chem-Biol. Interact.* 130–132: 761–773
- Ford G & Ellis EM (2001) Three aldo-keto reductases of the yeast *Saccharomyces cerevisiae*. *Chem-Biol. Interact.* 130–132: 685–698
- Ford G & Ellis EM (2002) Characterization of Ypr1p from *Saccharomyces cerevisiae* as α -methylbutyraldehyde reductase. *Yeast* 19: 1087–1096
- Forrest GL & Gonzalez B (2000) Carbonyl reductase. *Chem-Biol. Interact.* 129: 21–40
- Günther H (1994) *La spectroscopie RMN*. Masson, Paris.
- Habrych M, Rodriguez S & Steward JD (2002) Purification and identification of an *Escherichia coli* beta-keto ester reductase as 2,5-diketo-D-gluconate reductase YqhE. *Biotechnol. Prog.* 18: 257–261
- Hyndman D, Bauman DR, Heredia VV & Penning TM (2003) The aldo-keto reductase superfamily homepage. *Chem-Biol. Interact.* 143–144: 621–631
- Howe R & Moore RH (1971) Microbiological reduction of 2-methyl-1,2-di-3-pyridyl-1-propanone (Metyrapone). *J. Med. Chem.* 14: 287–288
- Jez JM & Penning TM (2001) The aldo-keto reductase (AKR) superfamily: an update. *Chem-Biol. Interact.* 130–132: 499–525

- Jornvall H, Hoog J & Persson B (1999) SDR and MDR: completed genome sequences show these protein families to be large, of old origin, and of complex nature. *FEBS Lett.* 445: 261–264
- Kallberg Y, Oppermann U, Jornvall H & Persson B (2002) Short-chain dehydrogenase/reductase (SDR) relationships: A large family with eight clusters common to human, animal and plant genomes. *Protein Sci.* 11: 636–641
- Kita K, Kataoka M & Shimizu S (1999) Diversity of 4-chloroacetoacetate ethyl ester-reducing enzymes in yeasts and their application to chiral alcohol synthesis. *J Biosci Bioeng* 88: 591–598
- Maser E (1995) Xenobiotic carbonyl reduction and physiological steroid oxidoreduction – the pluripotency of several hydroxysteroid dehydrogenases. *Biochem. Pharmacol.* 49: 421–440
- Maser E & Bannenberg G (1994) 11 β -Hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds. *Biochem. Pharmacol.* 47: 1805–1812
- Maser E, Gebel T & Netter KJ (1991a) Carbonyl reduction of metyrapone in human liver. *Biochem. Pharmacol.* 42: 593–598
- Maser E & Netter KJ (1989) Purification and properties of a metyrapone reducing enzyme from mouse liver microsomes – this ketone is reduced by an aldehyde reductase. *Biochem. Pharmacol.* 38: 3049–3054
- Maser E & Netter KJ (1991b) Reductive metabolism of metyrapone by a quercitrin-sensitive ketone reductase in mouse liver cytosol. *Biochem. Pharmacol.* 41: 1595–1599
- Maser E & Oppermann UCT (1997) Role of type-I 11 β -hydroxysteroid dehydrogenase in detoxification processes. *Eur. J. Biochem.* 249: 365–369
- Matsuda T, Harada T, Nakajima N, Itoh T & Nakamura K (2000) Two classes of enzymes of opposite stereochemistry in an organism: one for fluorinated and another for non fluorinated substrates. *J. Org. Chem.* 65: 157–163
- Nagamine S, Horisaka E, Fukuyama Y, Maetani K, Matsuzawa R, Iwakana S & Asada S (1997) Stereoselective reductive metabolism of metyrapone and inhibitory activity of metyrapone metabolites, metyrapol enantiomers, on steroid 11 β -hydroxylase in the rat. *Biol. Pharm. Bull.* 20: 188–192
- Oppermann U, Filling C, Hult M, Shafqat N, Wu X, Lindh M, Shafqat J, Nordling E, Kallberg Y, Persson B & Jörnvall H (2003) Short-chain dehydrogenase-reductase (SDR): the 2002 update. *Chem-Biol. Interact.* 143–144: 247–253
- Oppermann U & Maser E (1996) Characterization of a 3 α -hydroxysteroid dehydrogenase/carbonyl reductase from the Gram-negative bacterium *Comamonas testosteroni*. *Eur. J. Biochem.* 241: 744–749.
- Oppermann U & Maser E (2000) Molecular and structural aspects of xenobiotic carbonyl metabolizing enzymes. Role of reductases and dehydrogenases in xenobiotic phase I reactions. *Toxicology* 144: 71–81
- Pospisil S, Sedmera P, Havlicek V & Tax J (1994) Production of 26-deoxymonensins A and B by *Streptomyces cinnamonensis* in the presence of metyrapone. *Appl. Environ. Microbiol.* 60: 1561–1564
- Poupin P, Truffaut N, Combourieu B, Besse P, Sancelme M, Veschambre H & Delort AM (1998) Degradation of morpholine by an environmental strain of *Mycobacterium* involves a cytochrome P450. *Appl. Environ. Microbiol.* 64: 159–165
- Roberts SM (Ed) (1997) *Preparative Biotransformations*. Wiley & Sons, Chichester – New York
- Zelinski T, Peters J & Kula MR (1994) Purification and characterization of a novel carbonyl reductase isolated from *Rhodococcus erythropolis*. *J. Biotechnol.* 33: 283–292